Non-invasive Detection of Tumor Cell Death - Comparison between FDG and Hyperpolarized 13C-labelled Pyruvate

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Objective: Tumor response to therapy is primarily assessed in the clinic by monitoring reductions in tumor size. However, PET measurements of the uptake of the glucose analog, 2-^{18}Fluoro-2-deoxy-glucose (^{18}FDG), have demonstrated their potential for detecting earlier responses to treatment in the clinic. We have shown recently that the uptake and metabolism of hyperpolarized [1-^{13}C]pyruvate in lymphoma-bearing animals can be used to detect response to treatment using ^{13}C magnetic resonance spectroscopy and spectroscopic imaging [1]. The aim of the present study was to compare this measurement with changes in [^{14}C]FDG uptake in murine lymphoma cells following drug treatment.

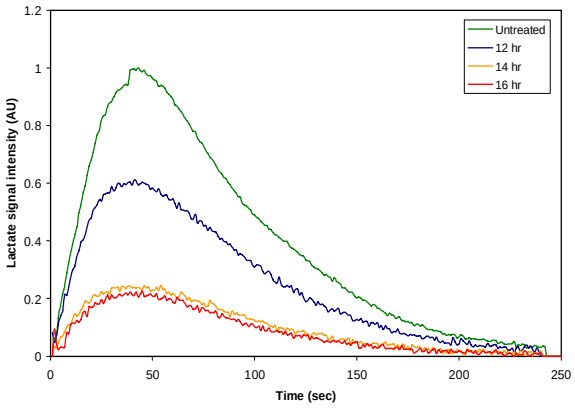

Fig 1. *^{13}C signal intensity from [^{13}C]lactate as a function of time following addition of 75mM hyperpolarized [1-^{13}C]pyruvate to a suspension of untreated EL-4 cells and cells treated with etoposide for the specified times.*

Methods: Murine lymphoma EL-4 cells were treated with 15 µM etoposide to induce apoptosis. Following treatment, [^{14}C]FDG uptake and hyperpolarized [1-^{13}C]pyruvate/[1-^{13}C]lactate flux were assessed every 2 hr over a 16 hr time course: 7.4 kBq [^{14}C]FDG was added to 2x10^6 cells and incubated for 60 minutes at 37°C. Samples were lysed in 0.2% SDS and FDG uptake was quantified by scintillation counting. 75 mM hyperpolarized [1-^{13}C]pyruvate [1,2] and 75 mM unlabelled lactate were added to 1x10^8 EL-4 cells in a 10 mm NMR tube, maintained at 37°C. ^{13}C spectra were acquired at 1 s intervals for 240 s using a low flip angle pulse in a 9.4 T vertical wide-bore magnet (Oxford Instruments). The exchange rate constants and spin lattice relaxation times were determined by fitting the peak intensities to the modified Bloch equations for two-site exchange [1]. The uptake of the fluorescent glucose analog, 2-NBDG, and changes in cellular NADH levels were assessed by flow cytometry using an LSR II cytometer (BD Biosciences), counting 20,000 cells per event. 1x10^6 cells were incubated with 100 µM 2-NBDG for 60 min prior to analysis, with changes in the NADH concentration assessed by measuring UV autofluorescence at 350nm. Apoptosis and necrosis were assessed by annexin V and SYTOX Red staining respectively.

Results and Discussion: Addition of 75 mM hyperpolarized [1-^{13}C]pyruvate to a suspension of untreated EL-4 cells resulted in detectable exchange of label with added lactate, in the reaction catalyzed by lactate dehydrogenase (LDH). Induction of apoptosis by the DNA-damaging agent, etoposide, led to a progressive decrease in the LDH-catalyzed flux between pyruvate and lactate (Fig. 1&2). This coincided with a loss of UV autofluorescence, from cellular NADH, which started to decrease 8 hours after drug treatment, a time at which the number of apoptotic cells increased markedly (Fig. 3). DNA damage activates Poly (ADP-Ribose) Polymerase (PARP), which leads to depletion of the intracellular NAD(H) pool [3]. After 16 hours the pyruvate/lactate flux was 30% of control. A decrease in [^{14}C]FDG uptake was observable as early as 6 hours after drug treatment and after 16 hours was 15% of the control value (Fig. 2). The decrease in glucose uptake was also assessed from measurements of 2-NBDG uptake (Fig. 3).

These data show that the decrease in lactate/pyruvate exchange coincides with early signs of cell death, and may therefore be useful for detecting early responses of tumors to chemotherapy. The loss of flux appears to be associated with the loss of the coenzyme NAD(H). The reduction in FDG uptake is also an early marker of cell death in this tumor model, showing a relatively rapid response following drug treatment.

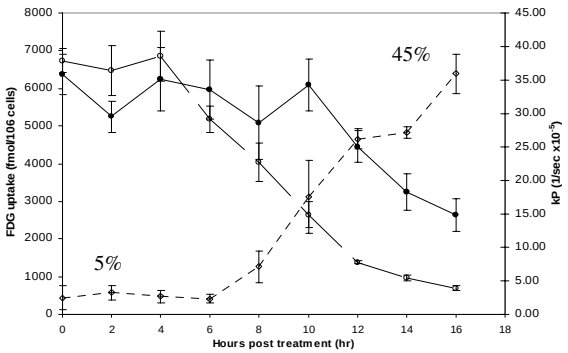

Fig 2. *Comparison of the decrease in [^{14}C]FDG uptake (open circles) with the decrease in ^{13}C label flux between pyruvate and lactate (closed circles) in etoposide-treated EL-4 cells as a function of time. The fraction of apoptotic cells are also shown (open diamonds). Mean values and standard errors are shown.*

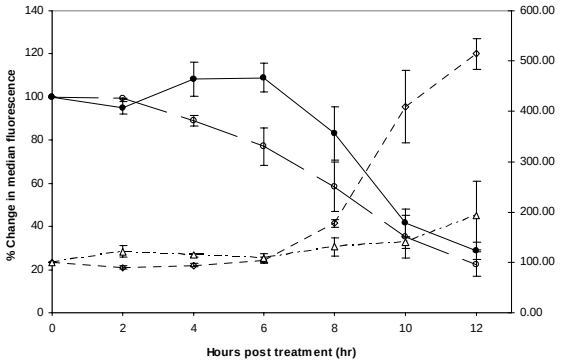

Fig 3. *Decrease in 2-NBDG uptake (open circle) and the intracellular NAD(H) pool (closed circle) in etoposide-treated EL-4 cells. The fraction of apoptotic (open diamond) and necrotic cells (open triangle) are also shown. Mean values and standard errors are shown.*

[1] Day SE et al. (2007) Nature Medicine. *In Press.* [2] Golman et al. (2006) Cancer Res. 66, 10855-10860.
[3] Williams SNO, Anthony ML, and Brindle KM (1998) Magn. Reson. Med. 40, 411-420.